PB84-170687

Monticello Experiment: A Case Study

Michigan State Univ., East Lansing

Prepared for

Environmental Research Lab.-Duluth, MN

1984

U.S. Department of Commerce
National Technical Information Service
NTIS

PB84-170687

EPA-600/D-84-067
1984

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by

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Grant 808845

EPA Project Officer
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OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
DULUTH, MN 55804

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TECHNICAL REPORT DATA *(Please read Instructions on the reverse before completing)*		
1. REPORT NO. EPA-600/D-84-067	2.	3. RECIPIENT'S ACCESSION NO. PB84 170687
4. TITLE AND SUBTITLE The Monticello Experiment -- A Case Study		5. REPORT DATE 1984 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) W. E. Cooper and R. J. Stout		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Michigan State University East Lansing, Michigan 48824		10. PROGRAM ELEMENT NO. 11. CONTRACT/GRANT NO. 808845
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Duluth, Minnesota 55804		13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA-600/3
15. SUPPLEMENTARY NOTES		

16. ABSTRACT

A number of assumptions are inherent in the process of using animal toxicity tests to determine water quality characteristics that can be considered safe for aquatic life. These assumptions, expressed as hypotheses, include: (1) the transfer of laboratory acute toxicity tests to a field situation is possible without serious distortion; (2) multispecies or community level responses to toxicity exposure will not be markedly different from single-species responses; and (3) intermittent exposures with short time intervals between events will produce the same ecological responses as a continuous exposure of the same concentration-time integral.

These hypotheses were tested in two recycling stream ecosystems at the Monticello Ecological Research Station of EPA's Duluth, Minnesota Environmental Research Laboratory. In testing the above hypotheses, population analyses of channel fish and benthic macroinvertebrate fauna plus laboratory toxicity tests on selected invertebrates from the channels were compared with evenness and diversity indices, measures of community metabolism, and leaf pack degradation for both continuous and pulsed para-cresol exposure regimes.

Conclusions were that (1) acute toxicity tests with fathead minnows, largemouth and smallmouth bass, damselfly larvae and amphipods produced estimated survivorship rates for exposure to p-cresol that were consistent with the field observations; (2) the structural and functional community parameters that were examined indicated the same type of ecological impacts on macroinvertebrates as did the single species analyses; and (3) the pulsed exposure produced a slightly greater impact than a continuous exposure of an equivalent concentration-time integral.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to public	unclassified	45
	20. SECURITY CLASS *(This page)*	22. PRICE
	unclassified	

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

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THE MONTICELLO EXPERIMENT - A CASE STUDY

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Introduction:

The use of animal toxicity tests to determine the water quality characteristics of permitted discharges generates an array of assumptions that need direct experimental verification. Issues involving the transfer of laboratory results to field situations, the sensitivity of test organisms compared to the natural residents, the ecological impacts of single-species versus multi-species responses and, finally, the independence of repeated insults versus additivity with increasing sensitivity are all critical research subjects. The E.P.A. (Environmental Protection Agency) outdoor experimental stream facility located at Monticello, Minnesota, and directed through the E.P.A. laboratory in Duluth, Minnesota represented a unique opportunity to test all of the above simultaneously. This paper reports on the effects of p-cresol application on the benthic macroinvertebrate fauna.

The experimental facility contains eight channels 500 m in length constructed with earthen bottoms. The channels consist of alternating 30 m long pools and riffles. Four channels were altered to create two isolated recycling stream ecosystems, each containing 1.5 million liters. The streams are physically, chemically and biologically typical of warm-water, grassland, third to firth order streams. The 1000 x 3 m channels were driven by recirculation pumps that propelled the stream water up to 11,000 1/m. Velocities could be run up to 12.2 cm/sec in the pools and up to 25 cm/sec in the riffles.

These two stream ecosystems were large enough and, owing to their history, were biologically diverse enough to be considered good analogs of natural streams. The only components of the biological community that were added were young-of-the-year smallmouth bass, largemouth bass, walleyed pike and fathead minnows. Fish were added to test ecological hypotheses concerning competitive displacements and to provide a vertebrate component to the species array for toxicity testing (Anderson, 1983).

Methods:

With the alteration of the channels to generate a recycling system, an application regime of the toxicant, p-cresol, could be generated that would hold a fixed exposure level for any given period of time. An onsite dedicated computer system monitored stream flows and was coupled with inputed p-cresol concentrations in the water so that it executed control over the toxicant injections to maintain approximate steady-state conditions. This system represents, therefore, a flowing, static test apparatus for acute toxicity testing (Cooper and Stout, 1982).

Several adjunct facilities were built to implement the laboratory anayses and testing. The computer facility was designed and operated by Dr. Robert Boling from Michigan State University. The organic chemistry laboratory was designed and operated by Dr. Robert Huggett from the Virginia Institute of Marine Sciences. The microcosm and bacteriological laboratory was designed and operated by Dr. Hap Pritchard from the E.P.A. Gulf Breeze Laboratory. The ecological experiments and field work were designed and implemented by Drs. Bill Cooper and Jean Stout from Michigan State University. The laboratory acute toxicity tests were performed by Dr. Don Mount at the E.P.A. Duluth Laboratory. Experiments of this type require the involvement of a truly interdisciplinary team and are rarely done. For this reason,

similar case studies will accumulate very slowly in the literature.

Details as to operating procedures and results of the microcosm experiments are being published elsewhere (Coleman et al., 1982; Pritchard et al., 1981; Anderson, 1983; Cooper and Stout, 1982; Stout and Cooper, 1983; Stout and Kilham, 1982). The effects of p-cresol on the benthic macro-invertebrates is the subject of this paper.

The field sampling was performed weekly, utilizing artificial substrate samplers for the riffles and a macrophyte sampler for the pools (Crowder and Cooper, 1982). Samples were washed through a 60 micron mesh Tyler seive and preserved in formalin, after which, the organisms were sorted, measured and identified to taxa. Means and variances were calculated by location and by date for population parameters (density, biomass, size dsitribution) and for community indices (number of species, diversity, evenness) calculated as the information measure of diversity and evenness (Pielou, 1969). The single species parameters were used to validate the laboratory-to-field transfer of the dose-response curves. The community indices are descriptive parameters which reflected the multi-species response to the toxicant.

Two other parameters reflect functional processes. Dissolved oxygen was measured continuously because it provides a measure of total community metabolism. Decomposition rates of leaves attached as packs to bricks were used to reflect organic carbon metabolism. Comparisons of population versus community responses were used to test for the presence of multispecies interactions. This is an essential issue in determining whether or not the extra effort and monies involved in multispecies testing is worth the commitment.

The experimental design involved four major p-cresol applications in the following pattern:

Duration	Channel	Date
24 hr Continuous	B	7/18/80 - 7/19/80
48 hr Continuous	A	8/9/80 - 8/11/80
96 hr Continuous	B	6/29/81 - 7/3/81
48 hr Pulsed	A	7/19/81 - 7/24/81

Each pair of channels followed its own seasonal pattern of population and community dynamics (Figs. 1 and 2). Within channel variation (Table I) represents microhabitat differences as there was no consistent trend from the headend (origin of pumping system) to tailend (turnaround area of channels) within the recycling system. The undosed channel for each experimental period was used as the reference channel. The species composition and life history patterns of the macroinvertebrates were similar for both channels. Assuming the community response would be correlated with the p-cresol addition, the companion channel, which at the time was not receiving p-cresol, was used to verify that no major shift, such as insect emergences or macrophyte dieoffs, was occurring.

TABLE I BENTHIC POOL SAMPLES, CHANNEL B, June 20, 1981

INTERPOOL VARIANCE (N = 2)

Location	Total Numbers	Total Biomass	Mean Biomass	Number of Spp.	Evenness	Diversity
Headend						
C_4P_2	1688	615.8	0.36	11	0.33	1.16
C_4P_6	1074	153.5	0.14	15	0.25	0.99
C_3P_6	1744	117.4	0.07	15	0.45	1.77
C_3P_6	1091	128.8	0.12	12	0.47	1.70
C_3P_4	1162	128.5	0.11	12	0.41	1.47
C_3P_4	779	133.3	0.17	17	0.42	1.71
C_3P_2	1475	280.9	0.19	15	0.26	1.03
C_3P_2	1993	502.5	0.25	13	0.39	1.44
Tailend						

P-cresol was added at a rate that would maintain ambient water concentrations of 8 ppm. This level was chosen as the best estimate from existing literature for a moderate toxic effect. The laboratory toxicity tests (Table II) confirms this estimate. The fish species showed a distinct "all or none" response pattern with no mortality at 10 ppm. The damselfly, Ischnura verticalis, showed no mortality response. The amphipod, Hyalella azetca, showed a more gradual dose pattern with mortality evident at 5 ppm at 96 hrs exposure time. Daphnia magna experienced mortality at all concentrations and exposure times. Since the field experiments involved an exposure concentrations of 8 ppm for duations of 24, 48 and 96 hrs, the amphipods should have experienced a moderate mortality and the fish and damselflies should have shown only behavioral effects.

The technology required to generate and maintain a dynamic system of this size at 8 ppm for periods up to 96 hrs required a massive effort. These developments have been previously reported by Boling, 1981; and Coleman, et al., 1982. Figure 3 from Cooper and Stout, 1982, presents the p-cresol concentrations during the 96 hr dose experiments at the head and tail ends of the recycling system.

An illustration of the complex dynamics that occur within a field system is provided by Figure 4 from Cooper and Stout, 1982. This figure presents the amount of p-cresol that was required to maintain the ambient concentrations illustrated in Figure 3. The diurnal oscillations reflect the changing rates of p-cresol degradation by bacteria in response to the diurnal cycle of dissolved oxygen. The four-day trend of increasing p-cresol additions reflected the adaptation of the bacterial community to the unique carbon

TABLE II

Dose-Response Data on P-Cresol With Selected Fish and Invertebrates From Monticello, Minn.

Mount and Norberg
E.P.A. Lab. at Duluth

Organism (number)	Initial Concentration of P-Cresol	TIME 24-hour % Survival	48-hour% Survival	72-hour % Survival	96-hour % Survival
Fathead minnow (10/run) 2 replicates	40 mg/l	60, 30	20, 0	0, 0	0, 0
	20 mg/l	60, 90	30, 40	0, 10	0, 0
	10 mg/l	100,100	100,100	100,100	100,100
	5 mg/l	100,100	100,100	100,100	100,100
	2.5 mg/l	100,100	100,100	100,100	100,100
	Control	100,100	100,100	100,100	100,100
Largemouth bass (5/run) 1 replicate	32 mg/l	0	-	-	-
	16 mg/l	100	100	100	100
	8 mg/l	100	100	100	100
	4 mg/l	100	100	100	100
	2 mg/l	100	100	100	100
	Control	100	100	100	100
Smallmouth bass (5/run) 1 replicate	40 mg/l	0	-	-	-
	20 mg/l	0	-	-	-
	10 mg/l	100	100	80	80
	5 mg/l	100	100	100	100
	2.5 mg/l	100	100	100	100
	Control	100	-	-	-
Largemouth bass Test #2 (5/run) 1 replicate	20 mg/l	0	-	-	-
	10 mg/l	100	100	100	100
	5 mg/l	100	100	100	100
	2.5 mg/l	100	100	100	100
	1.25mg/l	100	100	100	100
	Control	100	100	100	100

TABLE II, continued

Organism (number)	Initial Concentration of P-Cresol	TIME 24-hour% Survival	48-hour% Survival	72-hour% Survival	96-hour% Survival
Damselfly (10/run)	40 mg/l	-	100		
	20 mg/l	-	100		
1 replicate	10 mg/l	-	100		
	5 mg/l	-	100		
	2.5 mg/l	-	80		
	Control	-	70		
Amphipod *Hyalella azteca* (8/run)	80 mg/l	50,37.5	0, 0	0, 0	0, 0
	40 mg/l	87.5,50	25, 25	12.5,12.5	12.5,12.5
	20 mg/l	87.5,100	37.5,50	37.5,37.5	12.5,25
2 replicates	10 mg/l	100,100	75, 87.5	50, 50	37.5,50
	5 mg/l	100,100	87.5,87.5	87.5,50	75, 37.5
	Control	100,100	100,100	100,100	100,100
Waterflea *Daphnia magna* (5/run)	40 mg/l	0 , 0	0 , 0		
	20 mg/l	0 , 0	0 , 0		
	10 mg/l	0 , 0	0 , 0		
2 replicates	5 mg/l	60 , 80	60 , 40		
	2.5 mg/l	80 , 80	60 , 20		
	Control	100,100	100,100		

1. Flow per minute in chambers ranged from 21 to 60 ml/min.
2. Initial D.O. values ranged from 5.5 to 7.9 ppm
3. Final D.O. values ranged from 5.5 to 8.4 ppm
4. pH ranged from 7.2 to 7.5
5. Alkalinity= 40; hardness=46
6. Temperature was 24 to 26°C.
7. Measured concentration of p-cresol was done for each run. Those data are not presented here, but are part of the Mount and Norberg report to us and are available on request.

source. The control systems had to be on-line, dynamic, and with a short time lag in order to compensate for those transient behaviors.

Results:

Population Parameters:

The 24 hr dose produced no measurable effect on the populations (Figs. 1 and 2). The densities of individuals paralleled the total dry weight biomass; neither parameters indicated a reduction in populations in either the riffle or pool habitats as a result of p-cresol exposure.

The 48 hr dose resulted in varied behavioral effects on fish and on some invertebrates (Cooper and Stout, 1982). The responses ranged from death with walleyed pike, inhibition of feeding with largemouth base, no observable impacts on fathead minnows to observable mortality with amphipods and mayflies. The causal relationships are more complex than simply acute toxicity to target organisms. The p-cresol inhibited the photosynthetic activity of the filamentous algae. This, coupled with increasing respiration due to toxicant stress, generated a serious reduction in dissolved oxygen (Stout and Kilham, 1982). Levels of around 1 ppm were observed at 5:00 a.m. the morning at the end of the 48 hr dose.

The unexpected impact on photosynthetic activity generated a confounding effect of low dissolved oxygen stress on the acute toxicity testing of p-cresol. The data indicate a predominance of the low dissolved oxygen in that the less sensitive species to p-cresol also showed behavioral stress and

limited mortality rates. It is quite likely that the stress of p-cresol increased the sensitivity to low dissolved oxygen. Either way, the effect on the total biomass of benthic invertebrates was only moderate and the recovery was rapid (Figure 1).

The 96 hr dose produced a dramatic effect on both the population and community parameters. The total density of benthic invertebrates sharply declined in the pool habitat during the dose period (Figure 5). This time response was not as evident in the riffle habitat (Figure 6). The ambient concentrations of p-cresol were the same in both habitats. The most likely variant was the dissolved oxygen in specific microhabitats (Figure 7). The pools, with their macrophyte populations, have less mixing and a longer water turnover time than the riffle habitat. It is very likely that the dissolved oxygen reduction at night was much greater at the bottom of the pools and within the macrophyte beds than in the riffles. The dissolved oxygen probes were located in the pool habitats at a depth of approximately two feet. There are no microdistributional data available for dissovled oxygen, but the patterns are very suggestive that dissolved oxygen stress was most pronounced in the pools.

The total biomass of benthic invertebrates showed a similar trend of a much higher impact of p-cresol on the pool habitat as compared to the riffle habitat (Figs. 8 and 9). The amphipod, Hyalella azteca, was the most abundant macroinvertebrate in the benthos and macrophyte habitats (Table III). This organism also showed a high sensitivity to p-cresol (Table I). The reduction in biomass of Hyalella is obvious in Figure 10. The percentage surviving the

Mean Densities of Macroinvertebrates In

TABLE III BENTHIC SAMPLES, CHANNEL B, 1981

RIFFLE HABITAT*

Date	Chironomids	Physa	Hyalella	Leeches	Planaria	Isopods
6/20	26, 80	58, 7	1522,1396	13, 16	5, 1	36, 2
6/28	52, 82	56, 8	989, 744	2, 10	43, 5	23, 1
7/1	31, 61	38, 19	834,1143	44, 15	12, 2	30, 11
7/3	37, 19	84, 37	537, 198	28, 79	34, 4	96, 31
7/6	18, 20	118, 25	512, 786	22, 38	59, 0	22, 17
7/16	151,260	74, 7	442, 247	10, 7	21, 2	18, 3
7/21	62, 92	85, 28	350, 542	4, 12	34, 8	16, 6
7/26	35,165	59, 40	626, 400	12, 16	34, 6	58, 1
7/30	17,372	36, 62	682, 340	22, 31	110, 8	64, 7

*The pair of numbers is the mean density (N = 2) per sample for a riffle at the head end and the tail end of Channel B. Dose Period: 6/29 - 7/3

96 hr acute dose of 8 ppm is estimated from the laboratory data (Table I) to be approximately 50%. The survivorship rate observed in the field test was less than 10%. Further, the reduction in biomass, excluding Hyalella, ranged from 16 to 30% (Figure 11). Since many of these species are less sensitive to p-cresol, the indication is that low dissolved oxygen contributed appreciably to the reduction in benthic populations.

In an attempt to uncouple the low dissolved oxygen (secondary effect) from the acute toxicity of p-cresol (primary effect), we injected a pulsed dose whose integrated exposure (concentration x duration) approximated the same exposure as the continuous 48 hr experiment (Figure 12). A general reduction in total densities of macroinvertebrates was observed following the pulsed dose in both pools (Fig. 13) and riffles (Fig. 14). These reductions were more gradual than those observed in the pools during the 96 hr continuous application (Fig. 5). The dissolved oxygen was also reduced by the pulsed application but the recovery rate was more rapid than experienced during the 96 hr continous dose (Fig. 15).

The total biomass of both riffles (Fig. 16) and pools (Fig. 17) showed similar temporal trends. Again, the reduction in total biomass is not as striking as that observed in the pools during the 96 hr continuous dose (Fig. 8). Furthermore, there is no indication that the reductions in either biomass or density during this period are normal seasonal fluctuations (Fig. 1, 2 and 5).

The two macroinvertebrates that were tested in the laboratory were H. azetca and I. verticalis. The amphipod had an estimated survivorship of 80% after 48 hrs exposure at 8 ppm and the damselfly had 100% survivorship. These two species were the most abundant amphipod and damselfly in the field samples. Table IV presents the average density of Coenigrionidae in the pools and the one riffle that contained a significant population. The densities monotonically increased with the largest populations being present after mid-July. There was no indication of a reduction in damselfly densities associated with the pulsed injection of p-cresol.

The mean biomass of amphipods is presented in Table V. There was a reduction in amphipod biomass following the pulsed dose. The observed reduction depends upon which data one used for the baseline. The 16 July date was just prior to the initiation of the pulsed dose on 19 July. The July 26 date was the first date after the accumulated 8 ppm, 48 hr integrated exposure dose (Figure 12). If these two points are used, the percent survivorship was 66% for the pools and 77% for the riffles. If the July 30 date is utilized as endpoint, the estimated percentage survivorship is 31.6% for pools and 56.4% for riffles. These survivorship estimated still include the effects of both p-cresol toxicity and some low dissolved oxygen stress. The laboratory estimate was 80% survivorship (Table I). The field data appear to validate the laboratory estimates of survivorship when the acute toxicity of p-cresol was partially uncoupled from the secondary effect of low dissolved oxygen.

The comparison of the coupled and uncoupled effects can be further illustrated by comparing the reduction in amphipod biomass on 3 July in Channel B which received the 96 hr continuous dose with the period 19 July

TABLE IV. COENAGRIONIDAE, MEAN DENSITIES (1981) (N=2)

(Pulsed Dose, Channel A)

Date	Pools, A		Riffle, A	Pools, B	
	Tailend	Headend	Tailend	Tailend	Headend
6/20		0.5	0.0	1.0	1.0
6/28	9.5	2.5	0.0	2.0	4.0
7/1	10.0	8.5	0.5	1.0	1.0
7/3	4.5	0.5	2.5	1.0	1.5
7/6	2.5	7.0	6.5	0.0	1.5
7/16	48.0	25.5	7.0	15.0	8.5
7/21	23.5	45.0	9.5	7.5	13.0
7/26	16.0	46.0	3.5	12.0	1.5
7/30	18.0	10.5	2.5	15.5	17.5

TABLE V. AMPHIPODS, MEAN BIOMASS (1981)
N=4

Date	Channel A		Channel B	
	Pools	Riffles	Pools	Riffles
6/20	271.6	120.3	98.6	161.8
6/28	164.2	144.8	123.4	79.9
7/1	228.7	130.3	164.8	93.3
7/3	147.0	113.2	10.6	21.6
7/6	126.0	129.8	18.2	41.2
7/16	249.1	131.4	53.2	30.7
7/21	230.7	200.6	131.8	45.3
7/26	164.4	101.0	59.8	54.1
7/30	78.8	74.1	92.2	36.7

through 25 July in Channel A (Table III). The esitmated survivorship during the 96 hr dose was 8.6% in pools and 27.0% in riffles. The uncoupled effect of p-cresol on amphipods is also clearly illustrated in Table VI. The percentage composition of the macroinvertebrate biomass attributed to amphipods dropped sharply during the 96 hr dose and recovered within two weeks. No such shift in percenage composition appeared with the pulsed dose.

Community Parameters:

The community analyses are now presented as a comparison with the results of the population analyses. The community structure is measured as evenness and diversity indices. The community function is measured as total dissolved oxygen (already presented) and leaf pack degradation. To be comparable to the single specis analyses, the results should show no effect of the 24 hr and 48 hr doses in 1980. A strong effect of the 96 hr dose and a moderate effect of the pulsed dose should be seen in the 1982 data.

Table VII gives the mean evenness and diversity values for both habitats and channels for 1980. Channel A received the 48 hr dose from 9 August through 11 August. Channel B received the 24 hr dose from 19 July to 20 July. The pool habitat consistantly had a higher evenness and diversity throughout the summer. There are no discontinuities in either parameter associated with either temporal patterns or injection of p-cresol. This is consistant with the single species analyses.

Table VIII gives the mean evenness and diversity values for 1981, with Channel B receiving the 96 hr dose from 29 June to 3 July and Channel A receiving the pulsed dose from 19 July to 23 July. The 96 hr dose resulted

TABLE VI. AMPHIPODS, MEAN PERCENT BIOMASS (1981)*

N=4

Date	Channel A		Channel B	
	Pool	Riffle	Pool	Riffle
6/20	83.2	55.1	37.6	49.6
6/28	46.1	66.4	16.5	46.7
7/1	42.9	42.4	17.3	33.7
7/3	20.2	39.6	4.6	7.9
7/6	17.0	29.5	9.6	17.8
7/16	37.9	37.0	8.8	25.0
7/21	19.5	28.0	15.9	23.5
7/26	21.6	33.0	12.6	28.3
7/30	28.4	60.5	24.1	20.3

*The 96 hr continuous dose : Channel B, 6/29 - 7/3
The pulsed dose : Channel A, 7/19 - 7/24

TABLE VII. COMMUNITY PARAMETERS (EVENNESS, DIVERSITY), 1980

Mean Community Evenness (N=4)

Channel A				Channel B			
Date	Evenness POOLS	Date	Evenness RIFFLES	Date	Evenness POOLS	Date	Evenness RIFFLES
6/23	0.54	6/24	0.43	6/23	0.47	6/24	0.40
7/14	0.57	7/10	0.28	7/9	0.49	7/17	0.45
7/20	0.39	7/17	0.24	7/14	0.52	7/20	0.46
7/21	0.44	7/20	0.38	7/20	0.52	7/21	0.46
7/23	0.49	7/21	0.24	7/21	0.57	7/23	0.43
8/4	0.47	7/23	0.21	7/23	0.58	7/31	0.32
8/11	0.56	7/31	0.24	8/4	0.52	8/11	0.27
8/12	0.50	8/11	0.23	8/12	0.56	8/12	0.34
8/14	0.47	8/12	0.21	8/14	0.41	8/14	0.44
8/31	0.24	8/14	0.17	8/31	0.41	8/25	0.36
		8/25	0.28				
		9/7	0.38				

Mean Community Diversity (N=4)

Channel A				Channel B			
Date	Diversity POOLS	Date	Diversity RIFFLES	Date	Diversity POOLS	Date	Diversity RIFFLES
6/23	2.14	6/24	1.53	6/23	1.68	6/24	1.36
7/14	2.14	7/10	0.92	7/9	1.89	7/17	1.74
7/20	1.45	7/17	0.82	7/14	1.99	7/20	1.77
7/21	1.51	7/20	1.25	7/20	1.94	7/21	1.84
7/23	1.67	7/21	0.82	7/21	2.14	7/23	1.76
8/4	1.72	7/23	0.77	7/23	2.24	7/31	1.23
8/11	2.03	7/31	0.78	8/4	1.86	8/11	1.09
8/12	1.81	8/11	0.82	8/12	2.02	8/12	1.36
8/14	1.67	8/12	0.77	8/14	1.49	8/14	1.74
8/31	0.85	8/14	0.60	8/31	1.47	8/25	1.36
		8/25	0.96				
		9/7	1.26				

TABLE VIII. COMMUNITY PARAMETERS (EVENNESS, DIVERSITY), 1981

Mean Community Evenness (N=4)

Channel A				Channel B			
Date	Evenness POOLS	Date	Evenness RIFFLES	Date	Evenness POOLS	Date	Evenness RIFFLES
6/20	0.24	6/20	0.28	6/20	0.30	6/20	0.19
6/28	0.34	6/28	0.30	6/28	0.38	6/28	0.26
7/1	0.27	7/1	0.24	7/1	0.30	7/1	0.26
7/3	0.38	7/3	0.24	7/3	0.57	7/3	0.52
7/6	0.38	7/6	0.26	7/6	0.59	7/6	0.32
7/16	0.28	7/16	0.25	7/16	0.49	7/16	0.46
7/21	0.35	7/21	0.25	7/21	0.38	7/21	0.44
7/26	0.42	7/26	0.27	7/26	0.47	7/26	0.42
7/30	0.44	7/30	0.31	7/30	0.47	7/30	0.48

Mean Community Diversity (N=4)

Channel A				Channel B			
Date	Diversity POOLS	Date	Diversity RIFFLES	Date	Diversity POOLS	Date	Diversity RIFFLES
6/20	0.82	6/20	0.86	6/20	1.15	6/20	0.67
6/28	1.22	6/28	0.84	6/28	1.44	6/28	0.97
7/1	0.94	7/1	0.79	7/1	1.18	7/1	0.94
7/3	1.27	7/3	0.80	7/3	2.15	7/3	1.96
7/6	1.34	7/6	0.84	7/6	2.04	7/6	1.10
7/16	1.08	7/16	0.94	7/16	1.94	7/16	1.74
7/21	1.34	7/21	0.88	7/21	1.50	7/21	1.68
7/26	1.56	7/26	0.94	7/26	1.86	7/26	1.54
7/30	1.60	7/30	1.04	7/30	1.92	7/30	1.81

in a sharp increase in both community parameters. Neither of these recovered to their original values during the study.

The pulsed dose failed to generate a comparable response. There is a slight increase in evenness and diversity in the pools where the effects are expected to be a result of both acute toxicity of p-cresol and low dissolved oxygen. The community parameters again gave bascially the same results as the single species parameters. These results are understandable since the most abundant macroinvertebrate (Hyalella azteca) is also very sensitive to p-cresol and low dissolved oxygen. The disproportionate reduction of the dominant (Table VI) will affect both these parameters in the same fashion.

The remaining community parameter measures an aggregate process of leaf degradation. This experiment has been reported by Stout and Cooper (1983). Figure 18 presents the results measured as percent of dry weight biomass of poplar leaves for both the 96 hr and pulsed doses of p-cresol. There is a significant reduction in the degradation rate only during the period of the 96 hr dose. The processing function recovered rapidly. There is no significant reduction during the pulsed dose. These results are consistant with both the single species impacts and the community structure results.

Conclusions:

The Monticello experiment was designed to test several hypotheses. These are listed below accompanied with the conclusions that have resulted from the analysis of the ecological responses.

Hypothesis I: The transfer of laboratory acute toxicity tests to a field situation is possible without serious distortion.

Conclusion I: The acute toxicity tests with fathead minnows, largemouth bass, smallmouth bass, damselflies and amphipods produced estimated survivorship rates of exposure to p-cresol that were consistent with the results of the field experiments.

Hypothesis II: The results of single-species analyses will give similar results as will multispecies (community level) responses to exposure of p-cresol.

Conclusion II: The community parameters, both structural (evenness and diversity) and functional (community metabolism and leaf pack degradation), indicated the same type of ecological impacts on macro-invertebrates as did the single species analyses.

Hypothesis III: Pulsed exposures with short time intervals between events will produce the same ecological responses as a continuous exposure with the same integral of "exposure concentration x duration."

Conclusion III: The pulsed exposure produced slightly more impacts than continuous 48 hr exposure with a comparable "ppm x days" integral.

In addition to these anticipated impacts, some real ecological lessons resulted that were not previously anticipated. The major impact was not the primary impact of p-cresol on the macroinvertebrate community or on selected fish species. Rather, the major impacts were mediated through a primary effect of p-cresol on photosynthetic and respiration processes of aquatic plants. The secondary effects of low dissolved oxygen in addition to the acute toxicity of p-cresol generated striking impacts on both population and community parameters. Eventhough these impacts were rather severe, the populations and communities recovered over a period of several weeks.

The Monticello experience was a unique team effort that will be difficult

to repeat with any frequency. On the other hand, the results of direct field validation of more simple test systems are essential to the regulatory process. The Monticello facility is uniquely suited to this type of field toxicity testing. The scale, diversity and recycling characteristics of these stream ecosystems are essential for ecological validation experiments.

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LEGENDS FIGURES

Figure 1. Total mg dry weight biomass of macroinvertebrates from pools (-) and riffles (---) in Channel A for 1980. The vertical bars represent the range of duplicate samples. Riffle samplers included .050 m^2. Pool samplers included .042 m^2.

Figure 2. Total mg dry weight biomass of macroinvertebrates from pools (-) and riffles (---) in Channel B for 1980. The vertical bars represent the range of duplicate samples.

Figure 3. Ambient concentrations of p-cresol at the head (-•-) and tail (--) end of Channel B during the 96 hr continuous dose experiment, 1981.

Figure 4. Amount of p-cresol added to maintain the ambient concentrations during the 96 hr continuous dose experiment, 1981.

Figure 5. Numerical densities of macroinvertebrates in two pool habitats in Channel B, 1981. The pool samples included .042 m^2. Vertical bars represent the range of duplicate samples.

Figure 6. Numerical densities of macroinvertebrates in two riffle habitats in Channel B, 1981. The riffles samplers included .050 m^2. Vertical bars represent the range of duplicate samples.

Figure 7. Ambient dissolved oxygen levels in pool habitats in Channel B during the 96 hr continuous dose in 1981.

Figure 8. Mg dry weight biomass of macroinvertebrates in two pools habitats in Channel B, 1981. The pools samples included .042 m^2. Vertical bars represent the range of duplicate samples.

Figure 9. Mg dry weight biomass of macroinvertebrates in two riffle habitats in Channel B, 1981. The riffle samples included .050 m^2. Vertical bars represent the range of duplicate samples.

Figure 10. Mg dry weight biomass of amphipods in two pool habitats in Channel B, 1981. The pool samples included .042 m^2. Vertical bars repreent the range of duplicate samples.

Figure 11. Mg dry weight biomass of macroinvertebrates minus the amphipods in two pool habitats in Channel B, 1981. The pool samples included .042 m^2. Vertical bars represent the range of duplicate samples.

Figure 12. Ambient concentrations of p-cresol at the head (-•-) and tail (—) end of Channel A, 1981, during the pulsed dose.

Figure 13. Numerical densities of macroinvertebrates in two pool habitats in Channel A, 1981. The pool samples included .042 m^2. Vertical bars represent the range of duplicate samples.

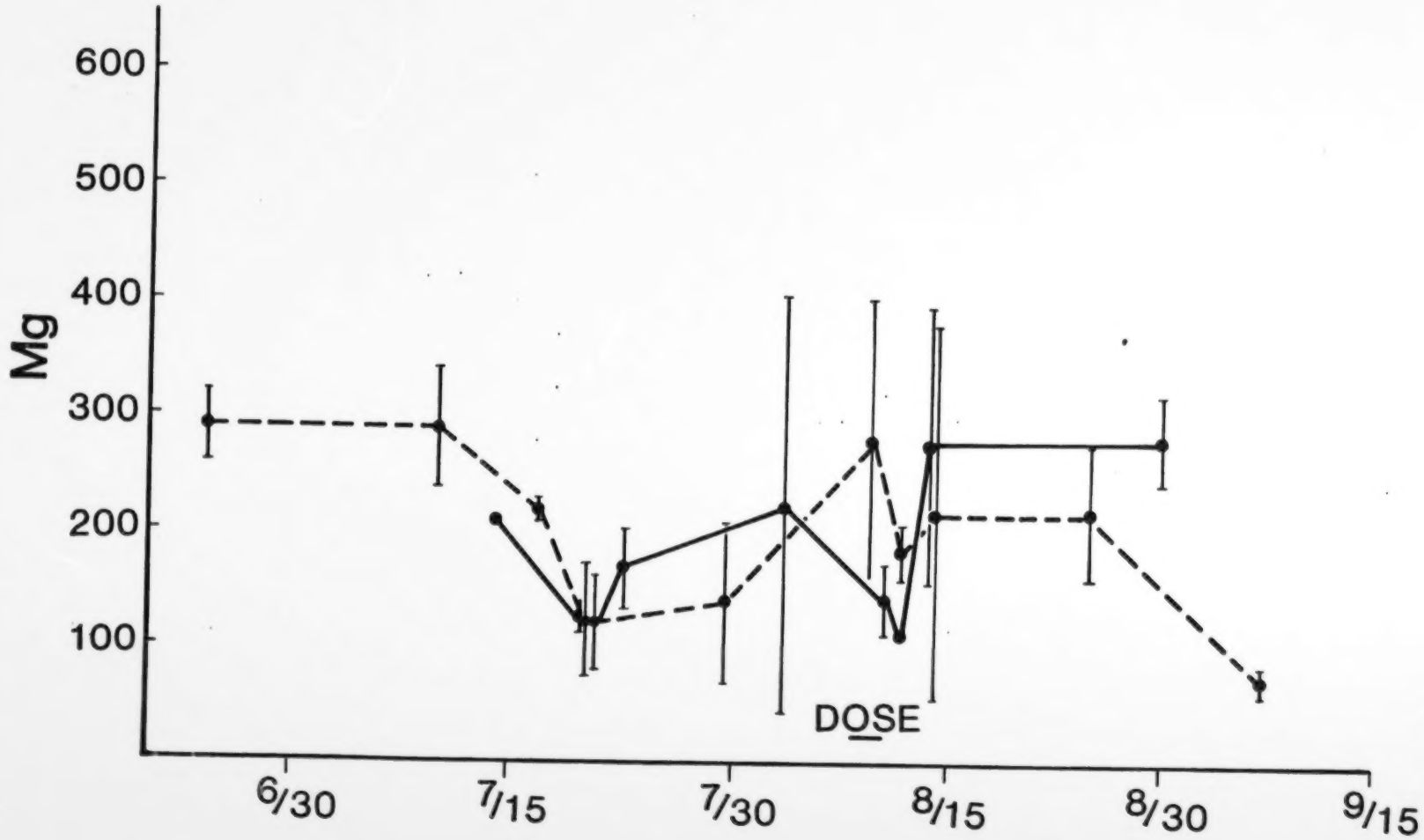
Figure 14. Numerical densities of macroinvertebrates in two riffle habitats in Channel A, 1981. The riffle samples included .050 m^2. Vertical bars represent the range of duplicate samples.

Figure 15. Ambient dissolved oxygen in the experimental (-) and control (-•-•-) channels during the pulsed dose.

Figure 16. Mg dry weight biomass of macroinvertebrates in two riffle habitats in Channel A, 1981. The riffle samples included .050 m^2. Vertical bars represent the range of duplicate samples.

Figure 17. Mg dry weight biomass of macroinvertebrates in two pool habitats in Channel A, 1981. The pool samples included .042 m^2. Vertical bars represent the range of duplicate samples.

Figure 18. Percent of original dry weight biomass of poplar leaves (Populus deltodies) remaining during the 96 hr and pulsed dose experiments in 1981.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Mg
DOSE
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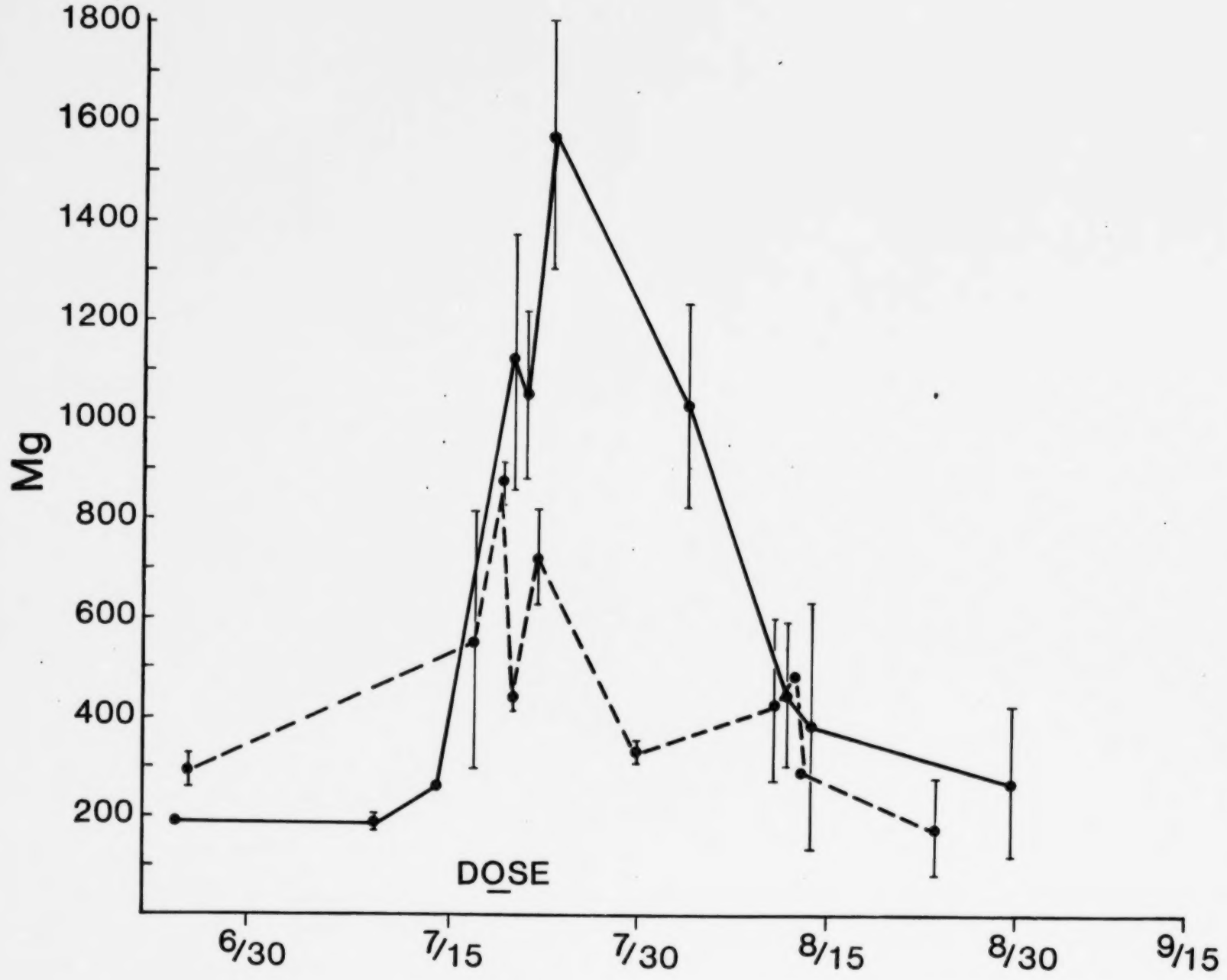
7/15
7/30
8/15
8/30
9/15


1800
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1400
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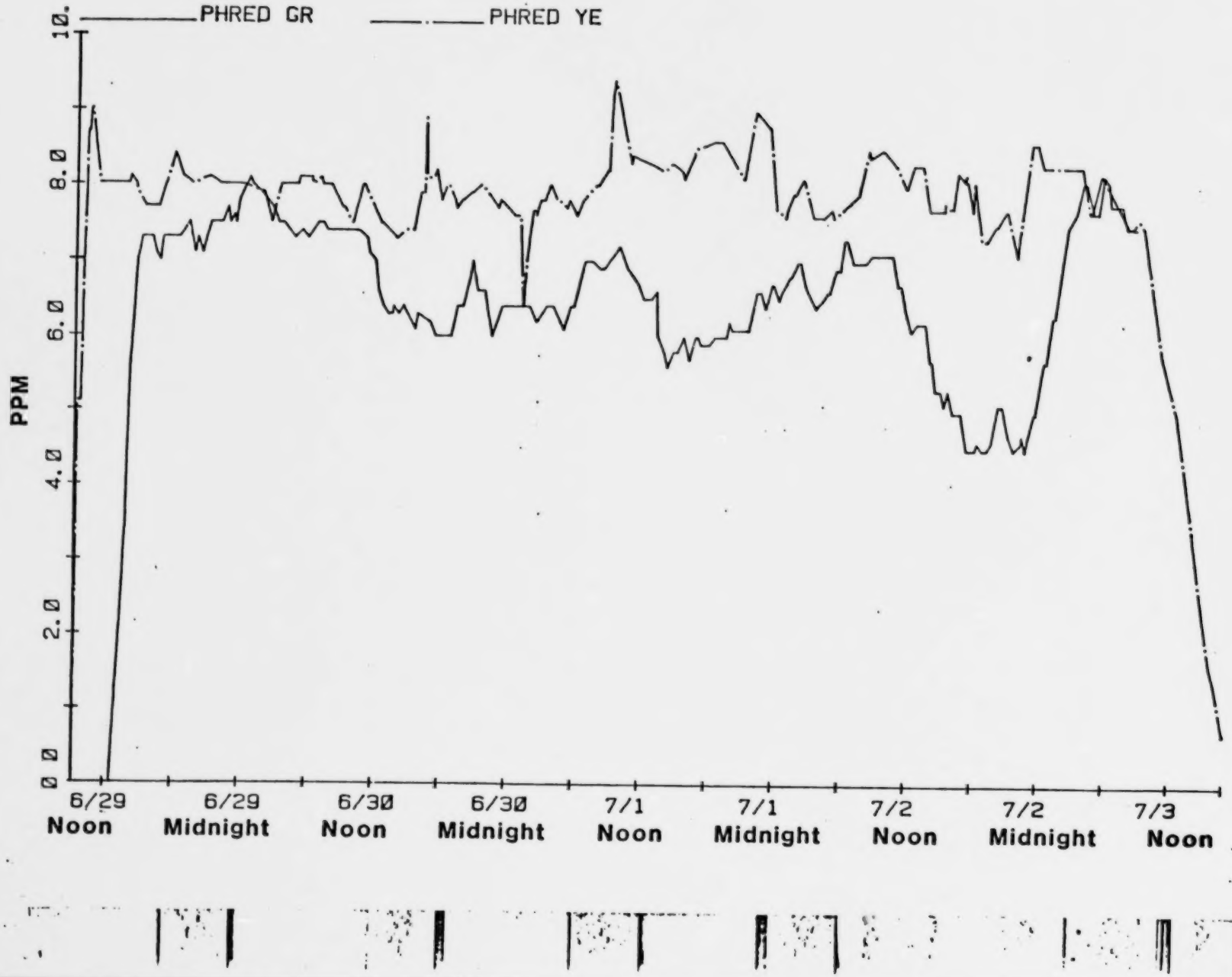
Mg
DOSE
6/30
7/15
7/30
8/15
8/30
9/15

3

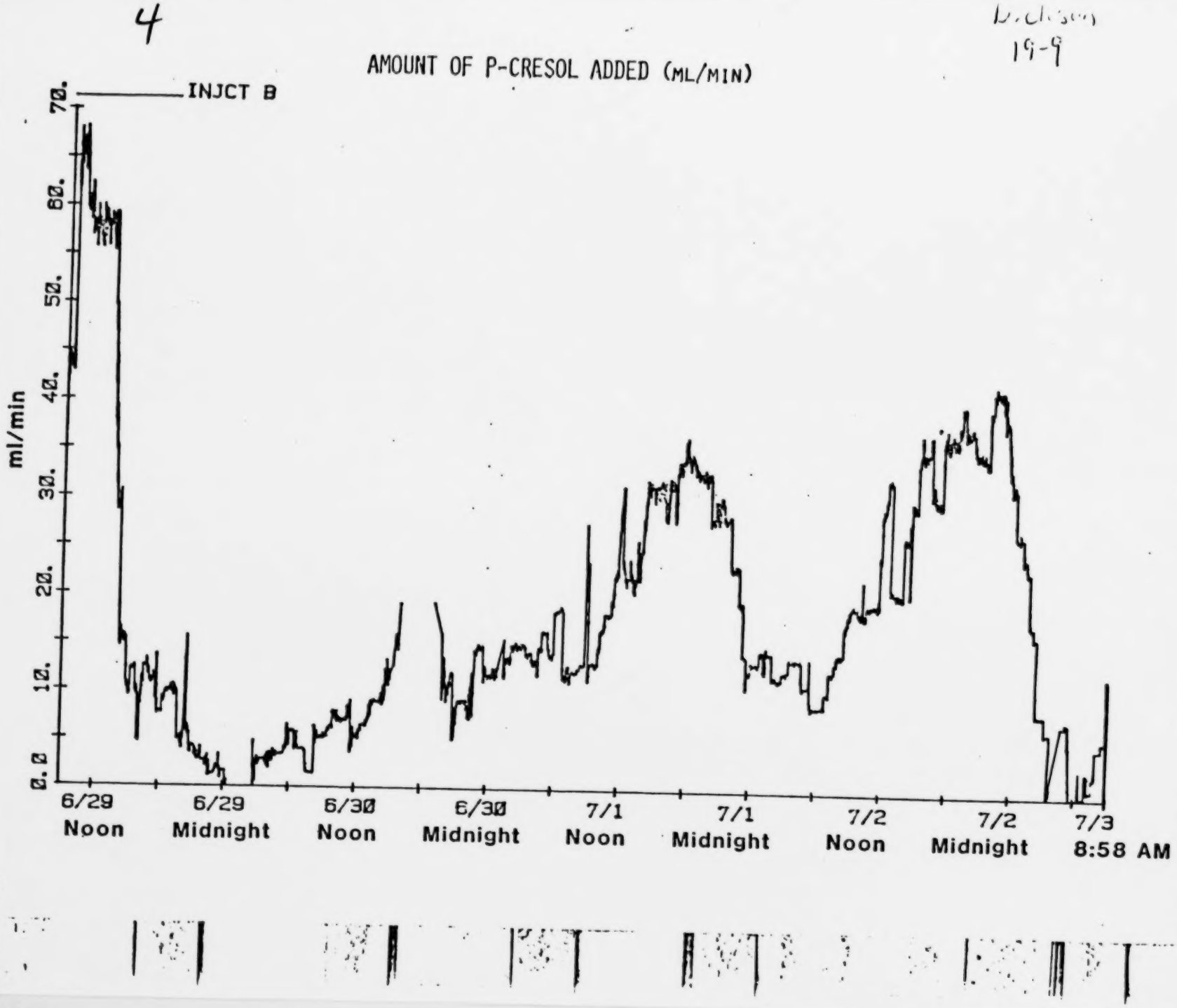
Dickson
19-8

AMBIENT CONCENTRATIONS OF P-CRESOL (PPM)



4

D. Olson
19-9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INJCT B
ml/min
70.
60.
50.
40.
30.
20.
10.
0.0
6/29 Noon
6/29 Midnight
6/30 Noon
6/30 Midnight
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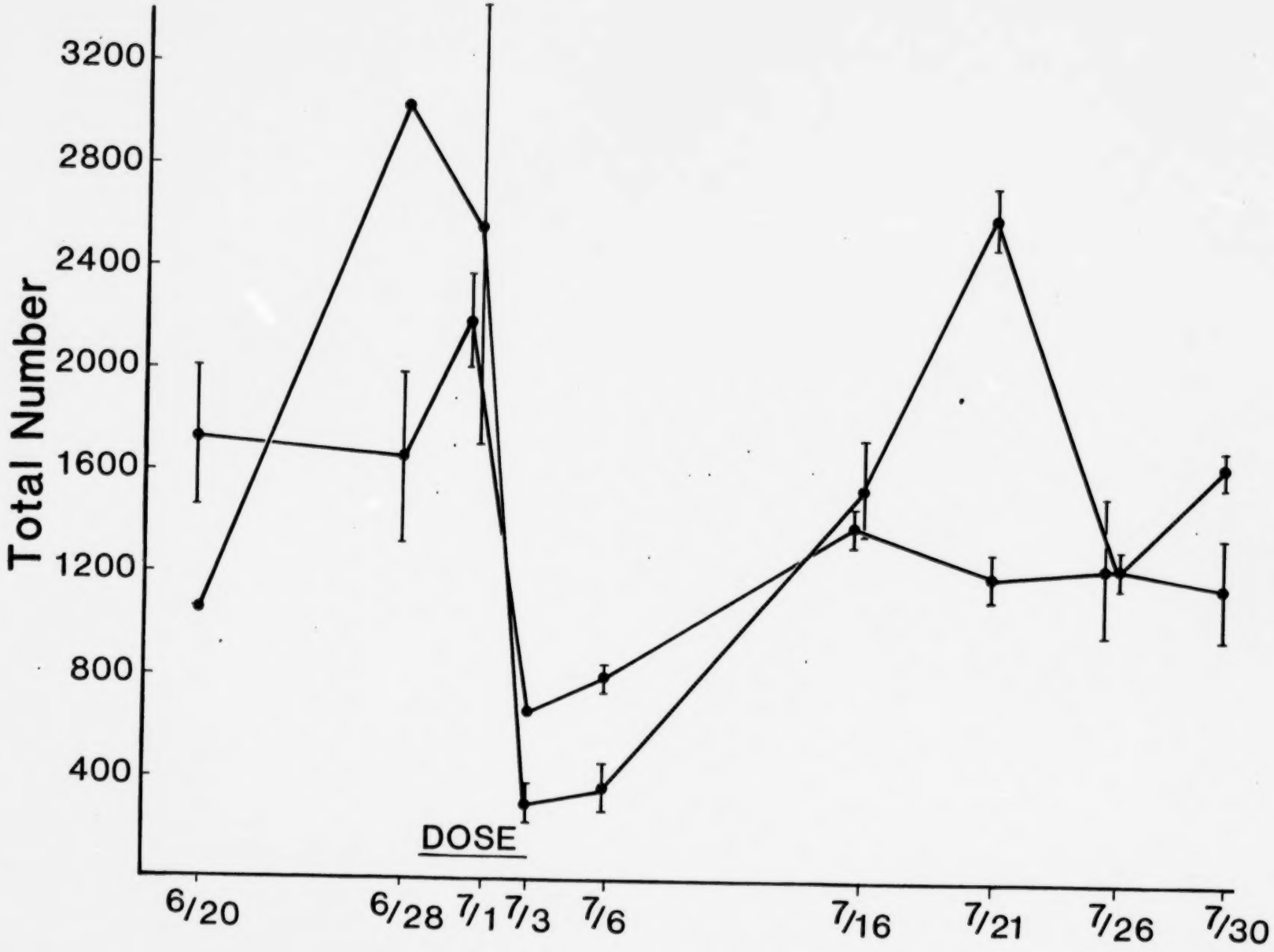
7/1 Midnight
7/2 Noon
7/2 Midnight
7/3 8:58 AM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2000
1600
1200
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DOSE
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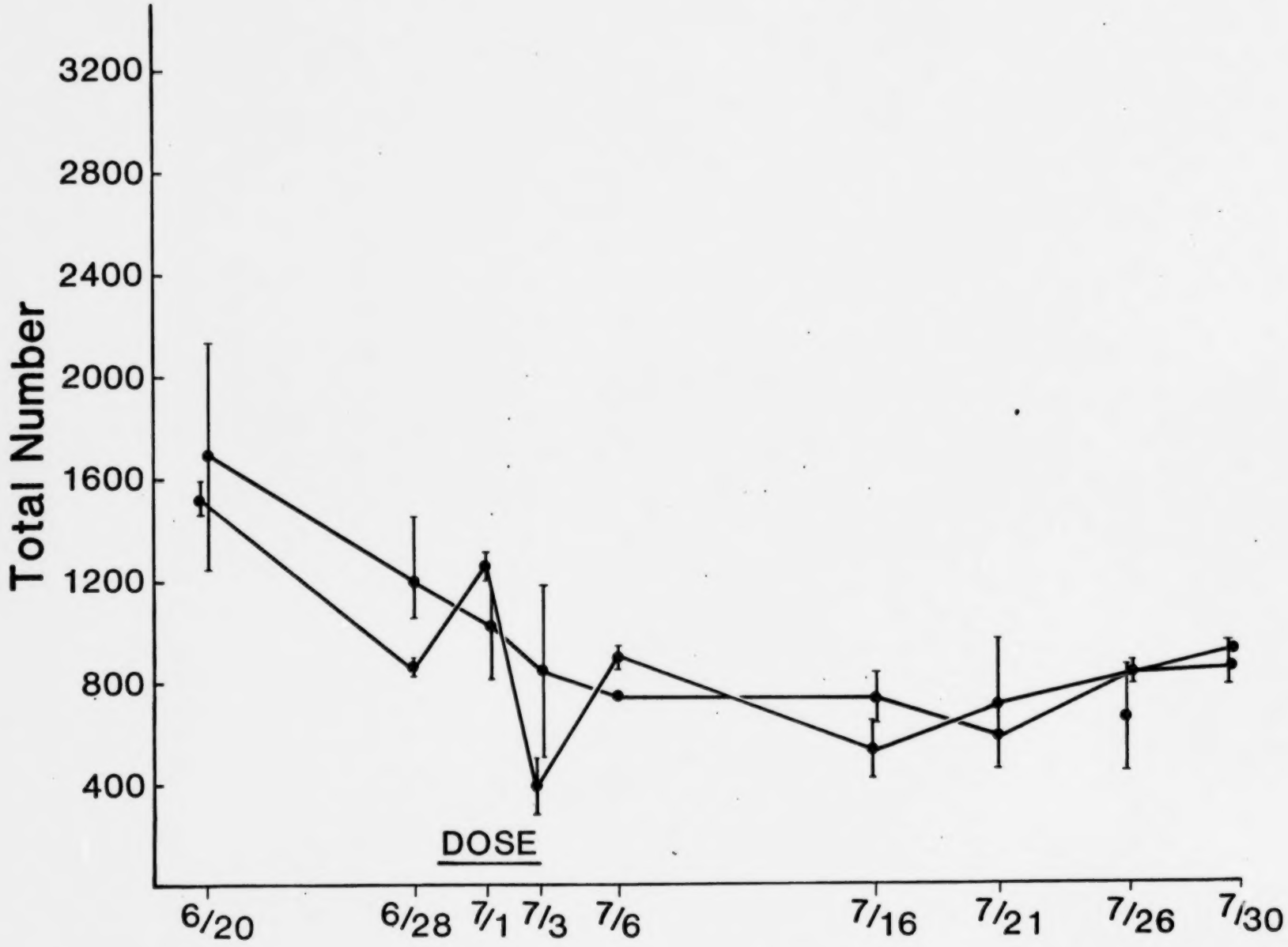
7/6
7/16
7/21
7/26
7/30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DOSE
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7/1
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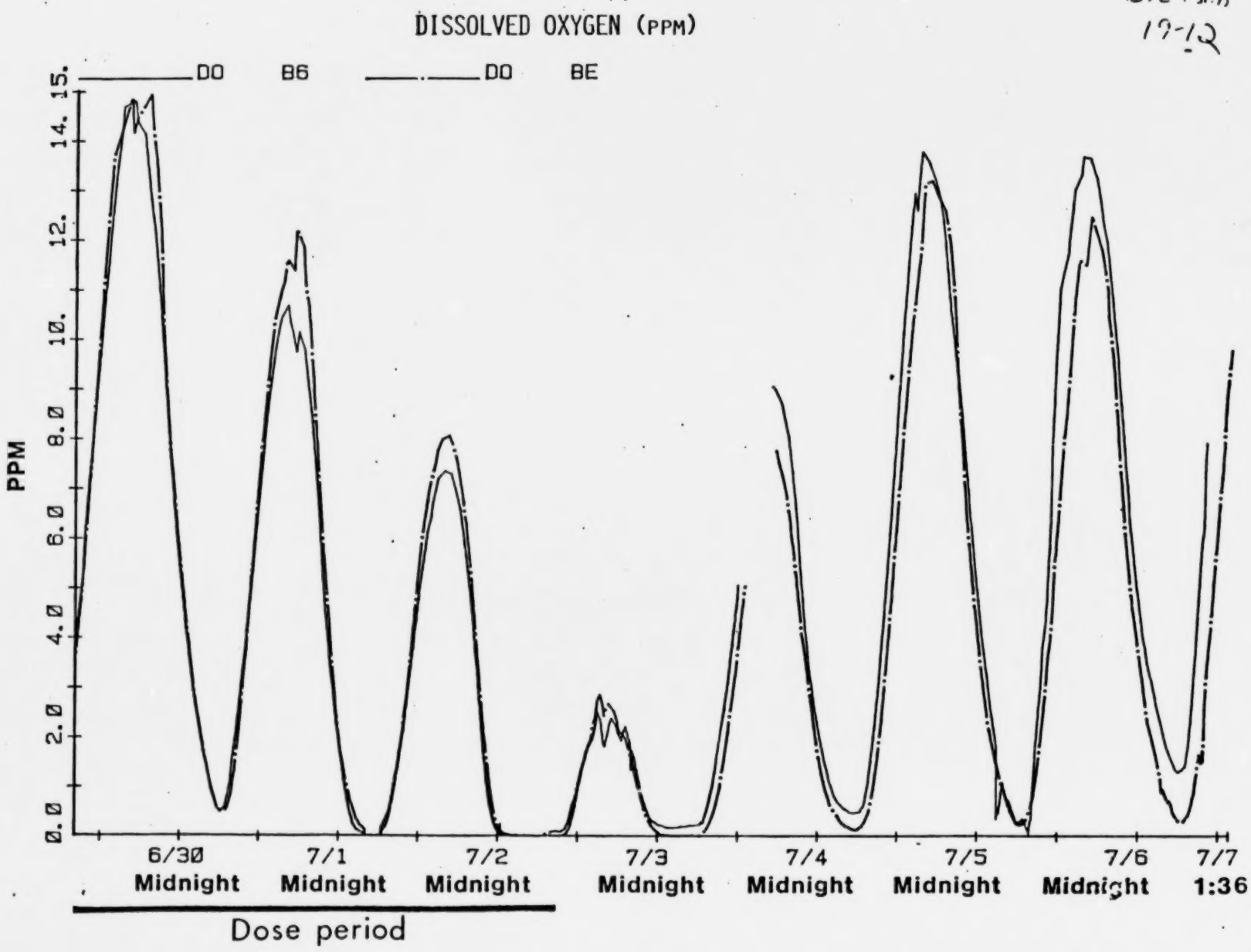
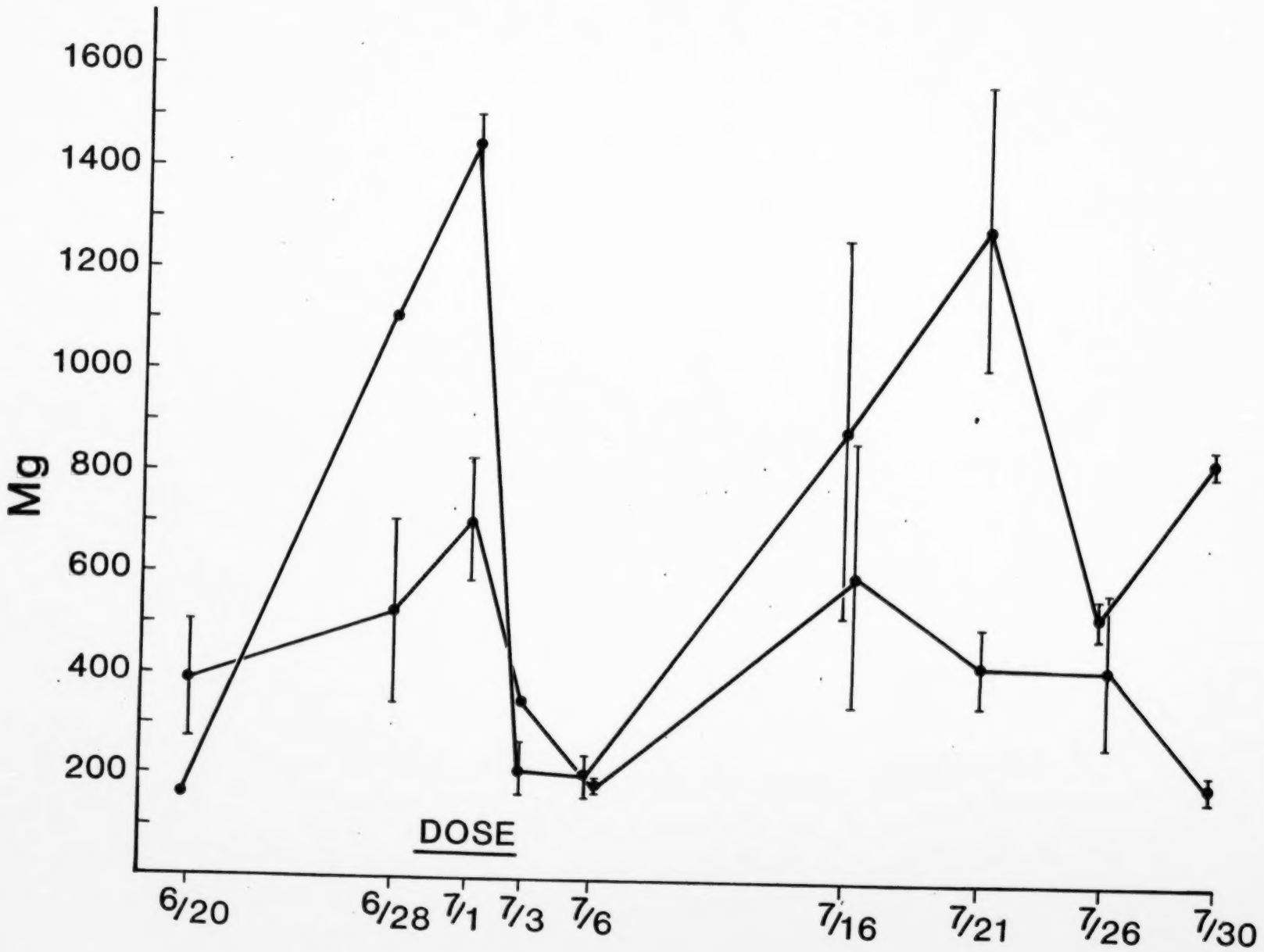
7/21
7/26
7/30

7

Dickson
19-12




1600
1400
1200
1000
800
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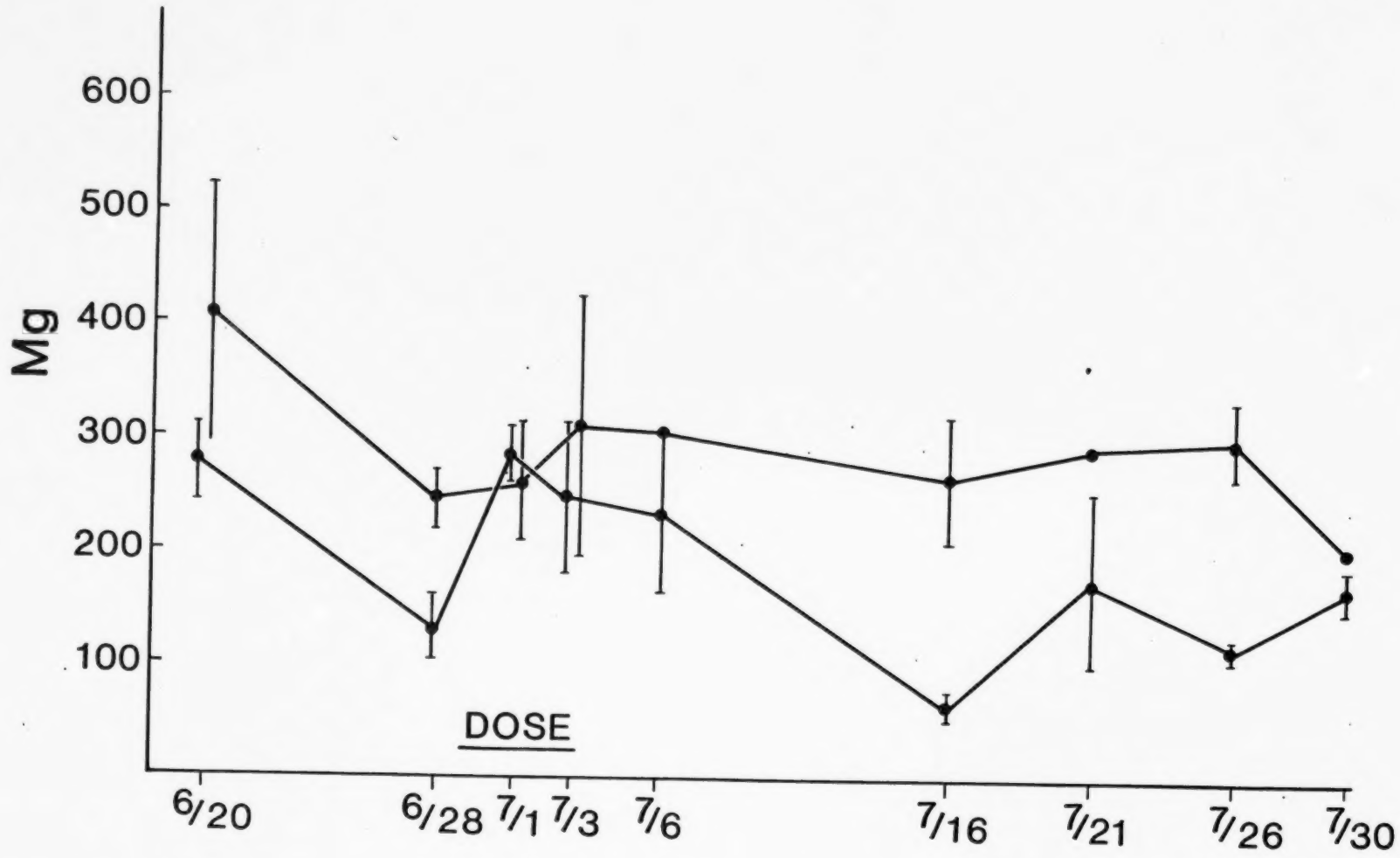
Mg
DOSE
6/20
6/28
7/1
7/3
7/6
7/16
7/21
7/26
7/30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500
400
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200
100
Mg
DOSE
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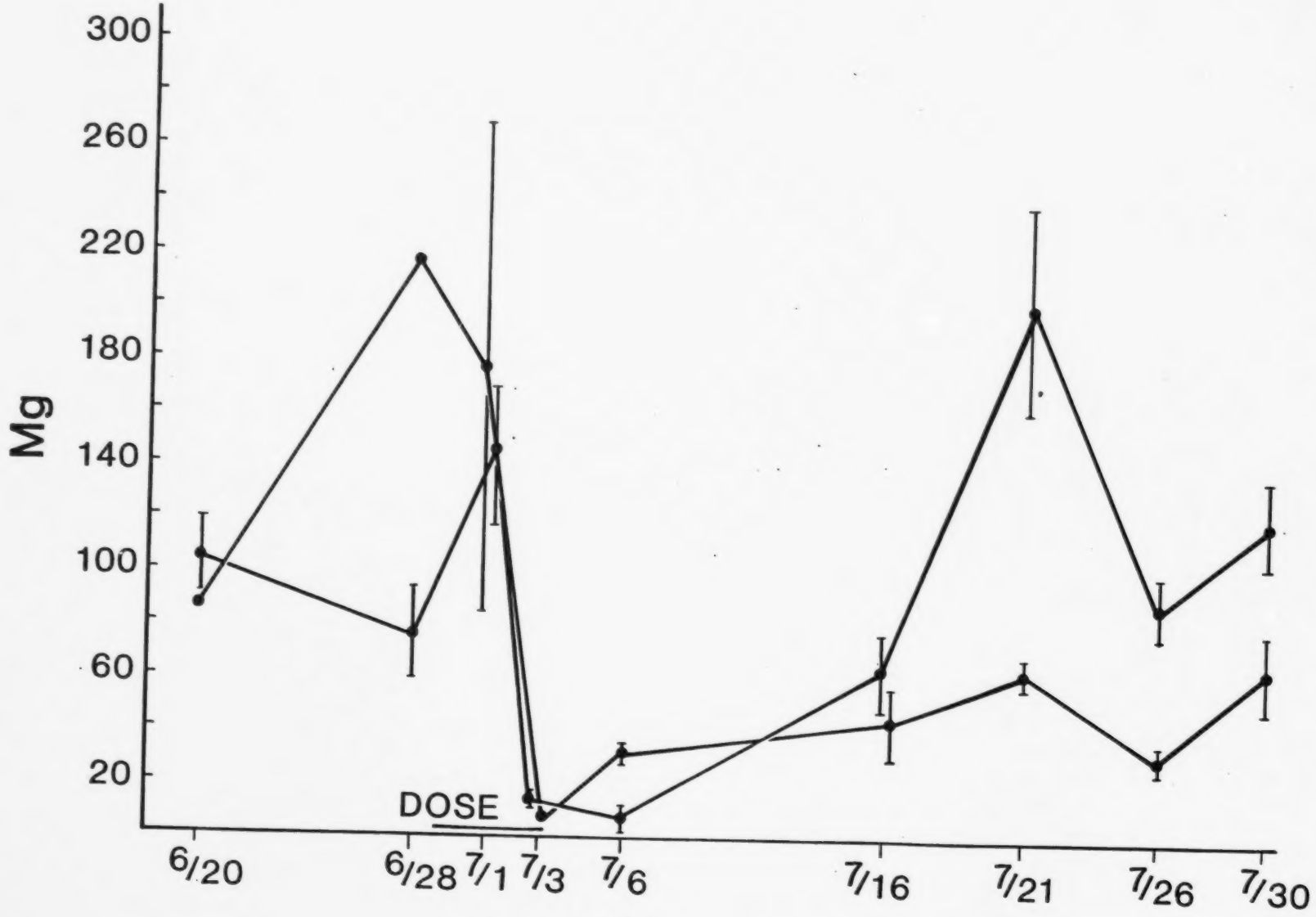
7/21
7/26
7/30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220
180
140
100
60
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Mg
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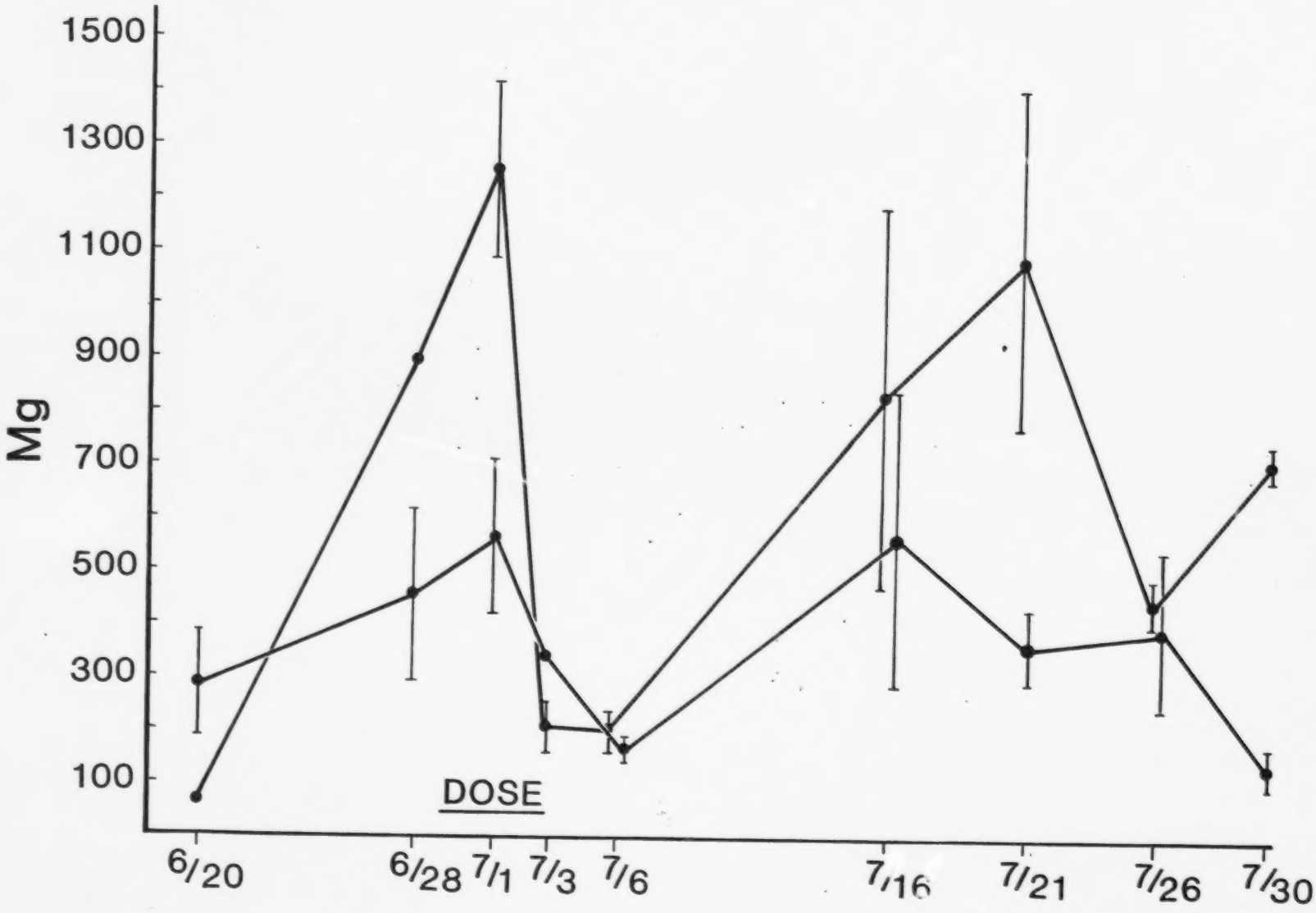
7/30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1300
1100
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100
Mg
DOSE
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6/28
7/1
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7/6
7/16
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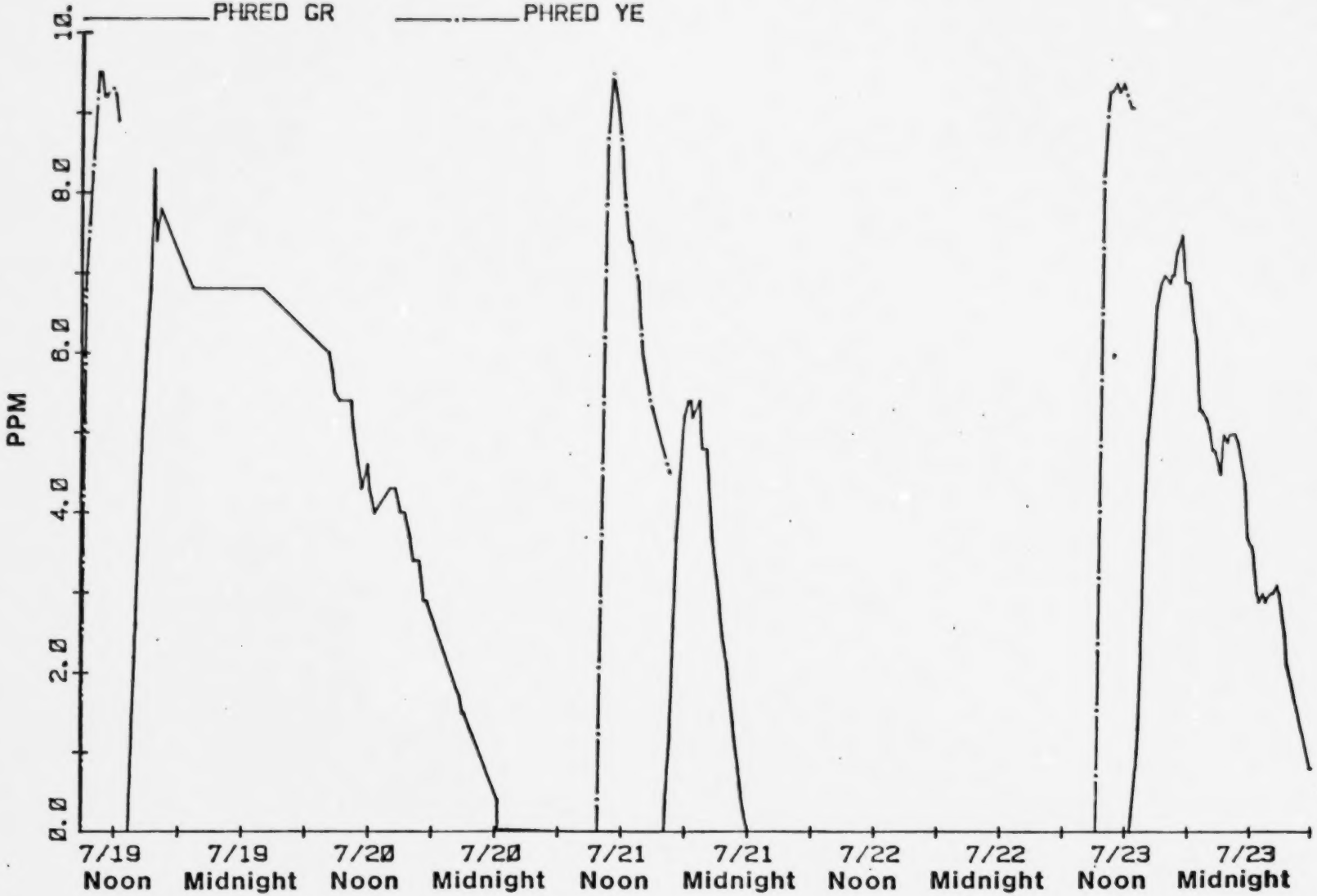
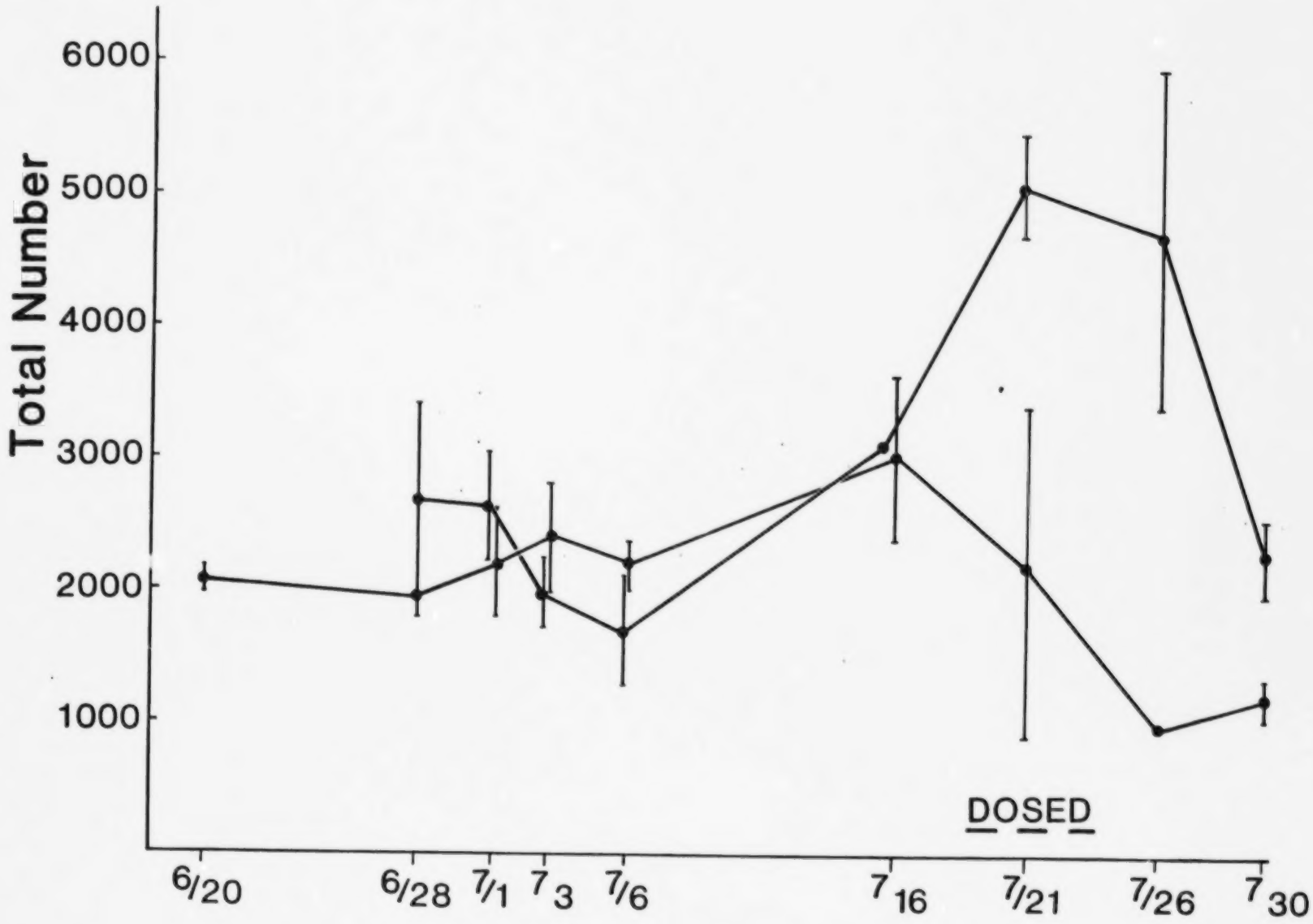
7/26
7/30

12

19-14

AMBIENT P-CRESOL CONCENTRATIONS (PPM)

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3000
2000
1000
DOSED
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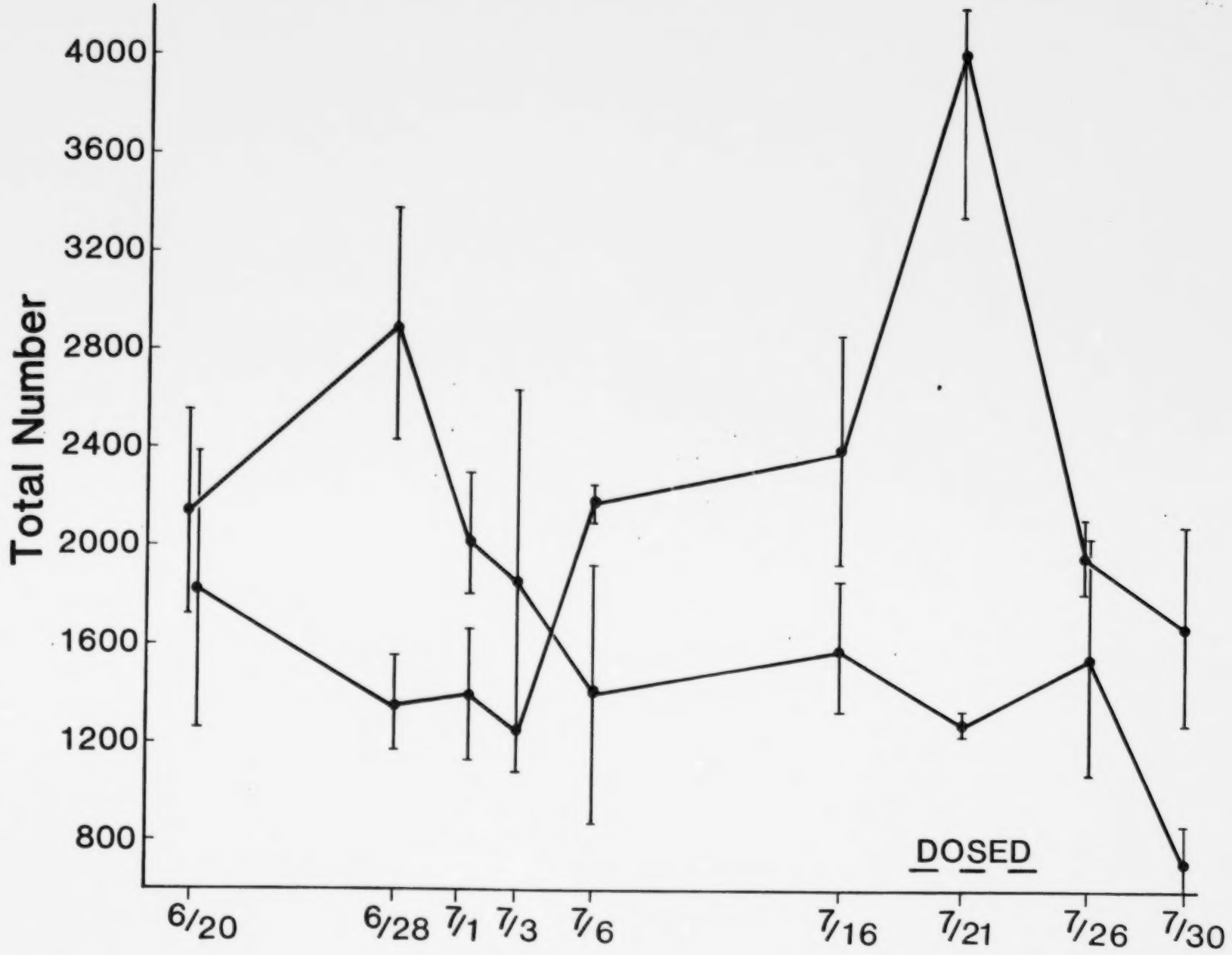
6/28
7/1
7 3
7/6
7 16
7/21
7/26
7 30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7/21
7/26
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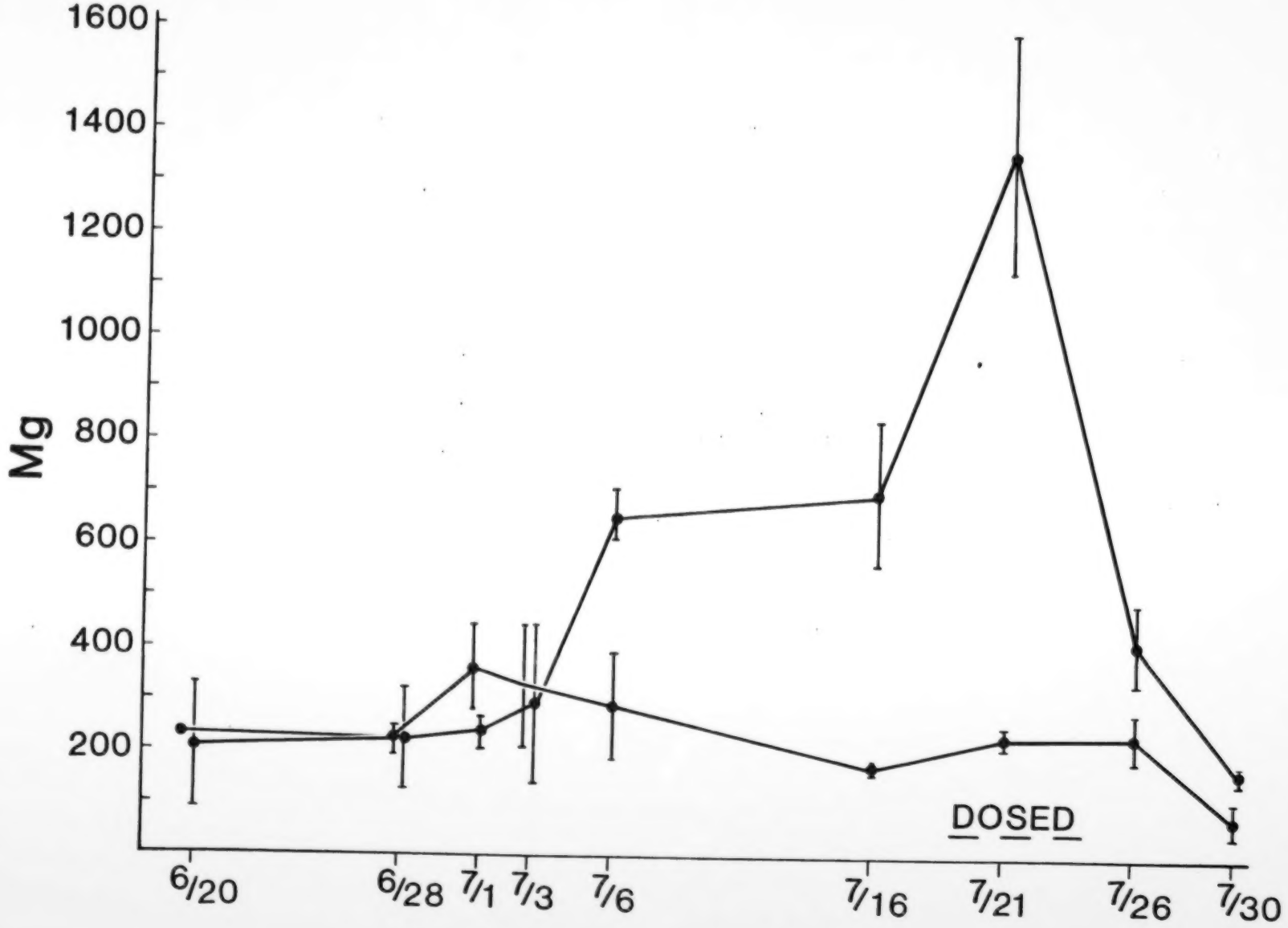
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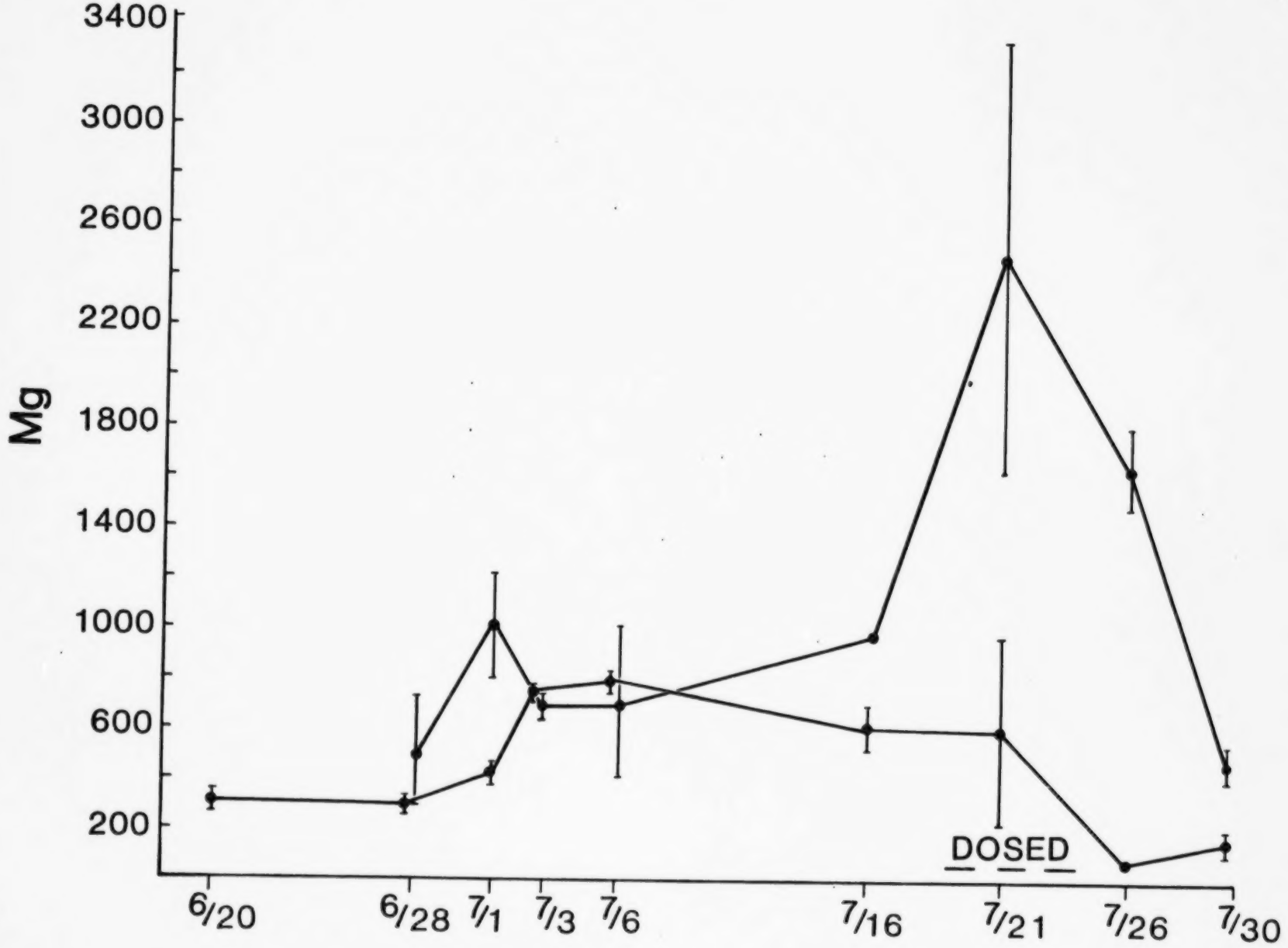
7/26
7/30


3400
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2200
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1400
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Mg
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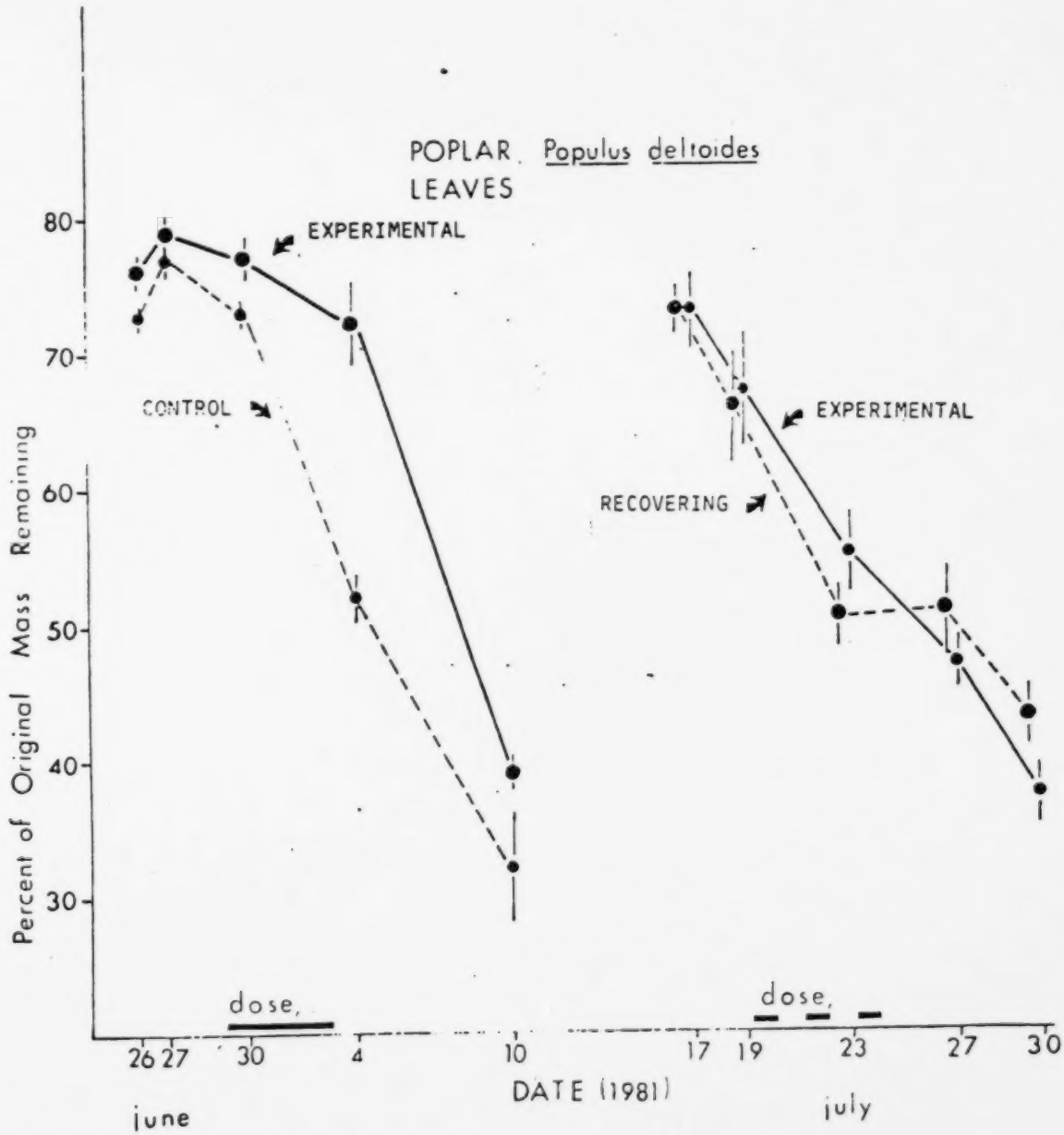
Fig


POPLAR Populus deltoides
LEAVES
EXPERIMENTAL
CONTROL
EXPERIMENTAL
RECOVERING
Percent of Original Mass Remaining
80
70
60
50
40
30
dose,
dose,
26 27
30
4
10
17
19
23
27
30
DATE (1981)
june
july